[00209] RT-PCR was performed as suggested by manufacturer using an EZ *rTth* RNA PCR kit (Perkins-Elmer, Foster City, CA). A typical reaction (50 Φl) contained 25-500 ng of total RNA, 100 ng of 5' target specific primer (common to *cis*- and *trans*-spliced products) (Lac-9F, 5'-GATCAAATCTGTCGATCCTTCC) (SEQ ID NO:82) and 100 ng of 3' primer (Lac-3R, 5'-CTGATCCACCCAGTCCCATTA, target specific primer for *cis*-splicing (SEQ ID NO:83), and Lac-5R, 5'-GACTGATCCACCCAGTCCCAGA (SEQ ID NO:84), PTM specific primer for *trans*-splicing), 1X reverse transcription buffer (100 mM Tris-HCl, pH 8.3, 900 mM KCL with 1 mM $MnCl_2$), 200 ΦM dNTPs and 10 units of *rTth* DNA polymerase. RT reactions were performed at 60EC for 45 min. followed by 30 sec pre-heating at 94EC and 25-35 cycles of PCR amplification at 94EC for 18 sec, annealing and extension at 60EC for 1 min followed by a final extension at 70EC for 7 min. The reaction products were analyzed by agarose gel electrophoresis.

REMARKS

Applicants submit herewith an initial Sequence Listing in computer and paper form, in accordance with 37 C.F.R. §1.821-1.825. The content of the paper and computer readable copies of the Sequence Listing submitted in accordance with 37 C.F.R. §1.821(c) and (e) are the same and do not include new matter. A copy of the Notice to Comply with Requirements for Patent Applications Containing Nucleotide Sequence and/or Amino Acid Sequence Disclosures is also enclosed.

The specification has been amended to insert sequence identifiers. No new matter has been introduced as a result of the amendments. Attached hereto is a page captioned

"VERSION WITH MARKINGS TO SHOW CHANGES MADE" indicating the changes made to the specification. Should any discrepancies be discovered, the version presented in the preceding "IN THE SPECIFICATION" section shall take precedence.

Please charge any additional fees associated with this filing or credit any overpayment to Deposit Account No. 02-4377. Two copies of this paper are enclosed.

Respectfully submitted,

Carmella L. Stephens

Rochelle K. Seide
PTO Registration No. 32,300

Carmella L. Stephens
PTO Registration No. 41,328
Attorney/Agent for Applicant

BAKER BOTTS, L.L.P.
30 Rockefeller Plaza
New York, NY 10112
(212) 408-2539

VERSION WITH MARKINGS TO SHOW CHANGES MADE

IN THE SPECIFICATION

Paragraph 0016 beginning on page 9, line 21 and ending on page 10, line 5 has been **amended** as follows:

[0016] Figure 3. Nucleotide sequence demonstrating the *in vitro trans*-spliced product between a PTM and target pre-mRNA (SEQ ID NO: 53). The 466 bp *trans*-spliced RT-PCR product from Figure 2 (lane 2) was re-amplified using a 5' biotin labeled forward primer (βHCG-F) and a nested unlabeled reverse primer (DT-3R). Single stranded DNA was purified and sequenced directly using toxin specific DT-3R primer. The arrow indicates the splice junction between the last nucleotide of target βHCG6 exon I and the first nucleotide encoding DT-A.

Paragraph 0017 on page 10 has been **amended** as follows:

[0017] Figure 4A. Schematic diagram of the "safety" PTM and variations, demonstrating the PTM intramolecular base-paired stem, intended to mask the BP and PPT from splicing factors (SEQ ID NOS: 54, 55, 56). Underlined sequences represent the βHCG6 intron 1 complementary target-binding domain, sequence in italics indicate target mismatches that are homologous to the BP.

Paragraph 0023 on page 11 has been **amended** as follows:

[0023] Figure 7B. Nucleotide sequence (sense strand) (SEQ ID NO:1) of the *trans*-spliced product between endogenous βHCG6 target and CRM197 mutant toxin is shown (SEQ ID NO: 57). Two arrows indicate the position of the splice junction.

Paragraph 0027 on page 12 has been **amended** as follows:

[0027] Figure 10A. Schematic diagram of constructs for use in the lacZ knock-out model. The target lacZ pre-mRNA contains the 5' fragment of lacZ (SEQ ID NO: 58 and SEQ ID NO: 67) followed by βHCG6 intron 1 (SEQ ID NO: 59 and SEQ ID NO: 68) and the 3' fragment of lacZ (SEQ ID NO: 60)(target 1). The PTM molecule for use in the model system was created by digesting pPTM +SP with PstI and HindIII and replacing the DT-A toxin with βHCG6 exon 2 (pc3.1PTM2).

Paragraph 0031 on page 13 has been **amended** as follows:

[0031] Figure 12A. Nucleotide sequence of *trans*-spliced molecule demonstrating accurate *trans*-splicing (SEQ ID NO: 61).

Paragraph 0032 on page 13 has been **amended** as follows:

[0032] Figure 12B. Nucleotide sequences of the *cis*-spliced product and the *trans*-spliced product (SEQ ID NOS: 62, 63). The nucleotide sequences were those sequences expected for each of the different splicing reactions.

Paragraph 0035 on page 13 has been **amended** as follows:

[0035] Figure 15. DNA sequence of the *trans*-spliced product (lane 1, lower band shown in Figure 14) (SEQ ID NO: 64). The DNA sequence indicates the presence of the F508 codon (CTT), exon 9 sequence is contiguous with exon 10 sequence, and the His tag sequence.

Paragraph 0042 on page 14 has been **amended** as follows:

[0042] Figure 22. Schematic diagram of mutant double splicing PTMs (SEQ ID NO:85).

Paragraph 0043 on page 14 has been **amended** as follows:

[0043] Figure 23. Accuracy of double-*trans*-splicing reaction (SEQ ID NOS:86, 87).

Paragraph 0051 on page 15 has been **amended** as follows:

[0051] Figure 31. PTM with a long binding domain masking two splice sites and part of exon 10 in a mini-gene target (SEQ ID NO:83).

Paragraph 0052 on page 15 has been **amended** as follows:

[0052] Figure 32. Sequence of a single PCR product showing target exon 9 correctly spliced to PTM exon 10 (with modified codons) (upper panel) (SEQ ID NO:89), codon 508 in exon 10 of the PTM (middle panel)(SEQ ID NO:90) and PTM exon 10 correctly spliced to target exon 11 (lower panel) (SEQ ID NO:91). The sequence of a repaired target was generated by RT-PCR followed by PCR.

Paragraph 0055 on page 15 has been **amended** as follows:

[0055] Figure 35. Schematic diagram of PTM exon 10 with modified codon usage to reduce antisense effects with its own binding domain (SEQ ID NO:92).

Paragraph 0056 on page 15 has been **amended** as follows:

[0056] Figure 36. Sequence of *cis*- and *trans*-spliced products (SEQ ID NOS:93, 94, 95, 96, 97).

Paragraph 0057 beginning on page 15, line 21 and ending on page 16, line 9 has been **amended** as follows:

[0057] Figure 37. Model system for repair of messenger RNAs by *trans*-splicing. (A) Schematic illustration of a defective lacZCF9m splice target used in the present study (see Materials and Methods for details). BP, branch point; PPT, polypyrimidine tracts; ss, splice sites and pA, polyadenylation signal (SEQ ID NO:98, 99). (B) A prototype PTM showing the key components of the *trans*-splicing domain (SEQ ID NO:100), and the diagrams of various PTMs showing the binding domain length and approximate positions at which they bind to the target pre-mRNA. Unique restriction sites within the *trans*-splicing domain are N, *Nhe* I; S, *Sac* II; K,

Kpn I and E, *EcoR* V. (C) Schematic diagram showing the binding of a PTM through antisense binding and repair of defective *lacZ* pre-mRNA through targeted RNA *trans*-splicing. Expected *cis* and *trans*-spliced products and the primer binding sites for Lac-9F, Lac-3R and Lac-5R are indicated.

Paragraph 0062 on page 18 has been **amended** as follows:

[0062] Figure 42. Complete sequence of CFTR PTM 30 (5' exon replacement PTM) showing the trans-splicing domain (underlined) (SEQ ID NO:102) and the coding sequence for exons 1-10 of the CFTR gene (SEQ ID NO:101). Modified codons in exon 10 are underlined and bold.

Paragraph 0063 on page 18 has been **amended** as follows:

[0063] Figure 43A. 153 base-pair PTM 24 Binding Domain (SEQ ID NO:103).

Paragraph 0064 on page 18 has been **amended** as follows:

[0064] Figure 43B. Complete sequence of CFTR PTM 24 (3' exon replacement PTM) showing the *trans*-splicing domain (underlined) (SEQ ID NO:104) and the coding sequence for exons 10-24 of the CFTR cDNA (SEQ ID NO:105). At the end of the coding is a histidine tag and the translation stop codon.

Paragraph 0065 on page 18 has been **amended** as follows:

[0065] Figure 44A. Detailed structure of the mouse factor VIII PTM containing normal mouse sequences for exons 16-26. BGH=bovine growth hormone 3' UTR (untranslated sequence); Binding Domain=125bp (SEQ ID NO:106); base changes to eliminate cryptic sites are circled:F5, F6, F7, F8=primer sites.

Paragraph 0067 on page 18 has been **amended** as follows:

[0067] Figure 44C. Changes to the promoter in AAV vectors pDLZ20 and pDLZ20-M2 to eliminate cryptic donor sites in sequence upstream of the PTM binding domain (SEQ ID NOS:108-109).

Paragraph 0069 on page 18 has been **amended** as follows:

[0069] Figure 45. Schematic diagram of a F8 PTM with the *trans*-splicing domain eliminated (SEQ ID NOS:110-111). This represents a control PTM to test whether repair is a result of *trans*-splicing.

Paragraph 0071 on page 19 has been **amended** as follows:

[0071] Figure 47A. Detailed structure of a mouse factor VIII PTM containing normal sequences for exons 16-26 and a C-terminal FLAG tag (SEQ ID NO:112). BGH=bovine growth hormone 3"UTR; Binding domain=125 bp.

Paragraph 0072 on page 19 has been **amended** as follows:

[0072] Figure 47B. Detailed structure of a human or canine factor VIII PTM containing normal sequences for exons 23-26 (SEQ ID NO:113).

Paragraph 0085 beginning on page 24, line 20 and ending on page 25, line 15 has been **amended** as follows:

[0085] A nucleotide sequence encoding a translatable protein capable of producing an effect, such as cell death, or alternatively, one that restores a missing function or acts as a marker, is included in the PTM of the invention. For example, the nucleotide sequence can include those sequences encoding gene products missing or altered in known genetic diseases. Alternatively, the nucleotide sequences can encode marker proteins or peptides which may be used to identify or image cells. In yet another embodiment of the invention nucleotide sequences encoding affinity tags such as, HIS tags (6 consecutive histidine residues) (Janknecht, et al.,

1991, Proc. Natl. Acad. Sci. USA 88:8972-8976), the C-terminus of glutathione-S-transferase (GST) (Smith and Johnson, 1986, Proc. Natl. Acad. Sci. USA 83:8703--8707) (Pharmacia) or FLAG (Asp-Tyr-Lys-Asp-Asp-Asp-Lys) (SEQ ID NO: 66) (Eastman Kodak/IBI, Rochester, NY) can be included in PTM molecules for use in affinity purification. The use of PTMs containing such nucleotide sequences results in the production of a chimeric RNA encoding a fusion protein containing peptide sequences normally expressed in a cell linked to the peptide affinity tag. The affinity tag provides a method for the rapid purification and identification of peptide sequences expressed in the cell. In a preferred embodiment the nucleotide sequences may encode toxins or other proteins which provide some function which enhances the susceptibility of the cells to subsequent treatments, such as radiation or chemotherapy.

Paragraph 0142 beginning on page 47, line 13 and ending on page 48, line 21 has been **amended** as follows:

[0142] Plasmids containing the wild type diphtheria toxin subunit A (DT-A, wild-type accession #K01722) and a DT-A mutant (CRM 197, no enzymatic activity) were obtained from Dr. Virginia Johnson, Food and Drug Administration, Bethesda, Maryland (Uchida *et al.*, 1973 J. Biol. Chem 248:3838). For *in vitro* experiments, DT--A was amplified using primers: DT-1F (5'-GGCGCTGCAGGGCGCTGATGATGTTGTTG) (SEQ ID NO:2); and DT-2R (5'-GGCGAAG CTTGGATCCGACACGATTTCCTGCACAGG) (SEQ ID NO:3), cut with PstI and HindIII, and cloned into PstI and HindIII digested pBS(-) vector (Stratagene, La Jolla, CA). The resulting clone, pDTA was used to construct the individual PTMs. (1) pPTM+: Targeted construct. Created by inserting IN3-1 (5'AATTCTCTAGATGCTT CACCCGGGCCTGACTCGAGTACTAACTGGTACCTCTTCTTTTTTTTCCTGCA) (SEQ ID NO:4) and IN2-4 (5'-GGAAAAAAAAGAAGAGGTACCAGTTAGTACTCGAGTCAGG

CCCGGGTGAAGCATCTAGAG) (SEQ ID NO:5) primers into EcoRI and PstI digested pDTA. (2) pPTM+Sp: As pPTM+ but with a 30 bp spacer sequence between the BD and BP. Created by digesting pPTM+ with XhoI and ligating in the oligonucleotides, spacer S (5'-TCGAGCAACGTTATAATAATGTTC) (SEQ ID NO:6) and spacer AS (5'-TCGAGAACATTATT ATAACGTTGC) (SEQ ID NO:7). For *in vivo* studies, an EcoRI and HindIII fragment of pcPTM+Sp was cloned into mammalian expression vector pcDNA3.1 (Invitrogen), under the control of a CMV promoter. Also, the methionine at codon 14 was changed into isoleucine to prevent initiation of translation. The resulting plasmid was designated as pcPTM+Sp. (3) pPTM+CRM: As pPTM+Sp but the wild type DT-A was substituted with CRM mutant DT-A (T. Uchida, et al., 1973, J. Biol. Chem. 248:3838). This was created by PCR amplification of a DT-A mutant (mutation at G52E) using primers DT-1F and DT-2R. For *in vivo* studies, an EcoRI HindIII fragment of PTM+CRM was cloned into pc3.1DNA that resulted in pcPTM+ARM. (4) PTM-: Non-targeted construct. Created by digestion of PTM+ with EcoRI and Pst I, gel purified to remove the binding domain followed by ligation of the oligonucleotides, IN-5 (5'-ATCTCTAGATCAGGCCCGGGTGAAGCC CGAG) (SEQ ID NO:8) and IN-6 (5'-TGCTTCACCC GGGCCTGATCTAGAG) (SEQ ID NO:9). (5) PTM-Sp, is an identical version of the PTM-, except it has a 30 bp spacer sequence at the PstI site. Similarly, the splice mutants [Py(-)AG(-) and BP(-)Py(-)AG(-)] and safety variants [PTM+SF-Py1, PTM+SF-Py2, PTM+SFBP3 and PTM+SFBP3-Py1] were constructed either by insertion or deletion of specific sequences (see Table 1).

Table 1 on page 49 has been **amended** as follows:

Table 1. Binding/non-binding domain, BP, PPT and 3' as sequences of different PTMs.

PTM construct	BD/NBD	BP	PPT	3'ss
PTM+Sp (targeted)	:TGCTTCACCCGGGCCTGA (SEQ ID NO:10)	TACTAAC	CTCTTCTTTTTTTTCC (SEQ ID NO: 11)	CAG
PTM-Sp (non-targeted)	:CAACGTTATAATAATGTT (SEQ ID NO:12)	TACTAAC	CTCTTCTTTTTTTTCC (SEQ ID NO:11)	CAG
PTM+Py (-)AG(-)BP(-)	:TGCTTCACCCGGGCCTGA (SEQ ID NO:10)	GGCTGAT	CTGTGATTAATAGCGG (SEQ ID NO: 13)	ACG
PTM+Py(-)AG(-)	:TGCTTCACCCGGGCCTGA (SEQ ID NO: 10)	TACTAAC	CCTGGACGCGGAAGT T (SEQ ID NO: 14)	ACG
PTM+SF	:CTGGGACAAGGACACTGCTT CACCCGGTTAGTAGACCACA GCCCTGAAGCC (SEQ ID NO: 15)	TACTAAC	CTTCTGTTTTTTTCTC (SEQ ID NO: 16)	CAG
PTM+SF-Py1	:As in PTM+SF	TACTAAC	CTTCTGTATTATTCTC (SEQ ID NO: 17)	CAG
PTM+SF-Py2	:As in PTM+SF	TACTAAC	GTTCTGTCCTTGTCTC (SEQ ID NO:18)	CAG
PTM+SF-BP3	:As in PTM+SF	TGCTGAC	CTTCTGTTTTTTTCTC (SEQ ID NO:16)	CAG
PTM+SFBP3-Py1	:As in PTM+SF	TGCTGAC	CTTCTGTATTATTCTC (SEQ ID NO: 17)	CAG

Paragraph 0147 beginning on page 50, line 18 and ending on page 51, line 16has been **amended** as follows:

[0147] RT-PCR analysis was performed using EZ-RT PCR kit (Perkin-Elmer, Foster City, CA). Each reaction contained 10 ng of *cis*- or *trans*-spliced mRNA, or 1-2 μg of total mRNA, 0.1 μl of each 3' and 5' specific primer, 0.3 mM of each dNTP, 1X EZ buffer (50 mM

bicine, 115 mM potassium acetate, 4% glycerol, pH 8.2), 2.5 mM magnesium acetate and 5 U of r*Tth* DNA polymerase in a 50 µl reaction volume. Reverse transcription was performed at 60°C for 45 min followed by PCR amplification of the resulting cDNA as follows: one cycle of initial denaturation at 94°C for 30 sec, and 25 cycles of denaturation at 94° C for 18 sec and annealing and extension at 60°C for 40 sec, followed by a 7 min final extension at 70°C. Reaction products were separated by electrophoresis in agarose gels.

Primers used in the study were as follows:

DT-1F: GGCGCTGCAGGGCGCTGATGATGTTGTTG (SEQ ID NO: 19)

DT-2R: GGCGAAGCTTGGATCCGACACGATTTCCTGCACAGG (SEQ ID NO: 20)

DT-3R: CATCGTCATAATTTCCTTGTG (SEQ ID NO: 21)

DT-4R: ATGGAATCTACATAACCAGG (SEQ ID NO: 22)

DT-5R: GAAGGCTGAGCACTACACGC (SEQ ID NO: 23)

HCG-R2: CGGCACCGTGGCCGAAGTGG (SEQ ID NO: 24)

Bio-HCG-F: ACCGGAATTCATGAAGCCAGGTACACCAGG (SEQ ID NO: 25)

β- globulin-F: GGGCAAGGTGAACGTGGATG (SEQ ID NO: 26)

β- globulin-R: ATCAGGAGTGGACAGATCC (SEQ ID NO: 27)

Table 2 on page 62 has been **amended** as follows:

Table 2. *Trans*-splicing in tumors in nude mice.

Mouse	Plasmid	Left Right	Electroporation	RT-PCR Left Right	Nested PCR	Nucleotide Sequence
[8]B1	pCMV-Sport	B1-1 B1-2	-	- -	- -	- -
B2	pCMV-Sport	B1-3 B1-4	[a]1000V/cm	- -	- -	- -
B3	pcSp+CRM	B3-1 B3-2	[a]1000V/cm	- -	- -	- -
		B3-3 B3-4	[a]1000V/cm	- -	- -	- -
B4	pcSp+CRM	B4-1 B4-2	[b]50V/cm	- -	- -	- -
		B4-3 B4-4	[c]25V/cm	- -	- -	- -
B5	pcSp+CRM/ pcHCG6	B5-1 B5-2	[a]1000V/cm	+ -	+ +	ATGTTCCAG↓GGCGTGATGAT [SEQ ID NO:53] (SEQ ID NO:65)
		B5-3 B5-4	[a]1000V/cm	+ -	+ +	ATGTTCCAG↓GGCGTGATGAT [SEQ ID NO:53] (SEQ ID NO:65)
B6	pcSp+CRM/ pcHCG6	B6-1 B6-2	[b]50V/cm	- -	- -	- -
		B6-3 B6-4	[c]25V/cm	- -	+ +	ATGTTCCAG↓GGCGTGATGAT [SEQ ID NO:53] (SEQ ID NO:65)
B7	pc PTM+Sp	B7-1	[a]1000V/cm	-	-	-
B8	pc PTM+Sp	B8-1	[b]50V/cm	-	%	ATGTTCCAG↓GGCGTGATGAT [SEQ ID NO:53] (SEQ ID NO:65)
[9]B9	pc PTM+Sp	B9-1	-	-	%	ATGTTCCAG↓GGCGTGATGAT [SEQ ID NO:53] (SEQ ID NO:65)

Paragraph 0165 on page 63 has been **amended** as follows:

[0165] The following primers were used for testing the *lacZ* model system:

5' Lac-1F GCATGAATTCGGTACCATGGGGGGGTTCTCATCATCATC (SEQ ID NO: 28)

5' Lac-1R CTGAGGATCCTCTTACCTGTAAACGCCCATACTGAC (SEQ ID NO: 29)

3' Lac-1F GCATGGTAACCCTGCAGGGCGGCTTCGTCTGGGACTGG (SEQ ID NO: 30)

3' Lac-1R CTGAAAGCTTGTTAACTTATTATTTTTGACACCAGACC (SEQ ID NO: 31)

3' Lac-Stop GCATGGTAACCCTGCAGGGCGGCTTCGTCTAATAATGGGACTGGGTG (SEQ ID NO: 32)

HCG-In1F GCATGGATCCTCCGGAGGGCCCCTGGGCACCTTCCAC (SEQ ID NO: 33)

HCG-In1R CTGACTGCAGGGTAACCGGACAAGGACACTGCTTCACC (SEQ ID NO: 34)

HCG-Ex2F GCATGGTAACCCTGCAGGGGCTGCTGCTGTTGCTG (SEQ ID NO: 35)

HCG-Ex2R CTGAAAGCTTGTTAACCAGCTCACCATGGTGGGGCAG (SEQ ID NO: 36)

Lac-TR1 (Biotin): 7-GGCTTTCGCTACCTGGAGAGAC (SEQ ID NO: 37)

Lac-TR2 GCTGGATGCGGCGTGCGGTCG (SEQ ID NO: 38)

HCG-R2: CGGCACCGTGGCCGAAGTGG (SEQ ID NO: 39)

Paragraph 0179 beginning on page 69, line 13 and ending on page 70, line 1 has been **amended** as follows:

[0179] The following oligonucleotides were used to create CFTR PTM:

Forward CF3

ACCT GGGCCC ACC CAT TAT TAG GTC ATT AT *CCGCGG* AAC ATT ATA
ApaI site. Intron 9 CFTR, -12 to -34. (SEQ ID NO: 40)

Reverse CF4
ACCT CTGCA*GGTGACC* CTG CAG GAA AAA AAA GAA G (SEQ ID NO: 41)
PstI BstEI PPT

Forward CF5
ACCT CTGCAG ACT TCA CTT CTA ATG ATG AT (SEQ ID NO: 42)
PstI. Exon 10 CFTR, +1 to +24

Reverse CF6
ACCT GCGGCCGC CTA ATG ATG ATG ATG ATG ATG CTC TTC TAG TTG GCA TGC
Not I. Stop Polyhistamine tag Exon 10 CFTR, +15 to +132 (SEQ ID NO: 43)

Paragraph 0180 beginning on page 70, line 2 and ending on page 71, line 1 has been **amended** as follows:

[0180] The following nucleotides were used to create the CFTR TARGET pre-mRNA mini gene (Exon 9 + mini-Intron 9 + Exon 10 + 5' end Intron 10):

Forward CF18
GACCT CTCGAG GGA TTT GGG GAA TTA TTT GAG (SEQ ID NO: 44)
XhoI Exon 9 CFTR, 1 to 21.

Reverse CF19
CTGACCT GCGGCCGC TAC AGT GTT GAA TGT GGT GC (SEQ ID NO: 45)
NotI. Intron 9 5' end.

Forward CF20
CTGACCT GCGGCCGC CCA ACT ATC TGA ATC ATG TG (SEQ ID NO: 46)
NotI. Intron 9 3' end.

Reverse CF21
GACCT CTTAAG TAG ACT AAC CGA TTG AAT ATG (SEQ ID NO: 47)
AflII Intron 10 5' end.

The following oligonucleotides were used for detection of trans-spliced products:

Reverse Bio-His
CTA ATG ATG ATG ATG ATG ATG (SEQ ID NO: 48)
Stop. Polyhistidine tag (5' biotin label).

Reverse Bio-His(2)
CGC CTA ATG ATG ATG ATG ATG (SEQ ID NO: 49)
3' UT Stop. Polyhistidine tag (5' biotin label).

Forward CF8
CTT CTT GGT ACT CCT GTC CTG (SEQ ID NO: 50)
Exon 9 CFTR.

Forward CF18
GACCT CTCGAG GGA TTT GGG GAA TTA TTT GAG (SEQ ID NO: 51)
XhoI. Exon 9 CFTR.

Reverse CF28
AAC TAG AAG GCA CAG TCG AGG (SEQ ID NO: 52)
Pc3.1 vector sequence (present in PTM 3' UT but not target).

Paragraph 0188 beginning on page 73, line 15 and ending on page 74, line 13 has been **amended** as follows:

[0188] The important structural elements of DSPTM7 (Figure 21) are as follows:

(1) 3' BD (120 BP) (SEQ ID NO:69):

GATTCACTTGCTCCAATTATCATCCTAAGCAGAAGTGTATATTCTTATT

TGTAAAGATTCTATTAACTCATTTGATTCAAAATATTTAAAATACTTCCTGTTT

CATACTCTGCTATGCAC

(2) Spacer sequences (24 bp) (SEQ ID NO:70): AACATTATTATAACGTTGCTCGAA

(3) Branch point, pyrimidine tract and acceptor splice site(SEQ ID NO:71):

3' ss

BP Kpn 1 PPT EcoRV ↓lacZ mini-exon

TACTAAC T GGTACC TCTTCTTTTTTTTTT GATATC CTGCAG |GGC GGC|

(4) 5' donor site and 2nd spacer sequence (SEQ ID NO:72):

5' ss

lacZ mini-exon ↓

|TGA ACG| GTAAGT GTTATCACCGATATGTGTCTAACCTGATTCGGGCCTTC

GATACGCTAAGATCCACCGG

(5) 5' BD (260 BP)(SEQ ID NO:73):

TCAAAAAGTTTTCACATAATTTCTTACCTCTTCTTGAATTCATGCTTTG

ATGACGCTTCTGTATCTATATTCATCATTGGAAACACCAATGATTTTTCTTTAA

TGGTGCCTGGCATAATCCTGGAAAACTGATAACACAATGAAATTCTTCCACT

GTGCTTAAAAAAACCCTCTTGAATTCTCCATTTCTCCCATAATCATCATTACA

ACTGAACTCTGGAAATAAAACCCATCATTATTAACTCATTATCAAATCACGC

Paragraph 00189 on page 74 has been **amended** as follows:

[00189] To determine whether the restoration of β-gal function is RNA *trans*-splicing mediated, the mutants are depicted in Figure 22. DSPTM8 is a 3' splice mutant in which the 3' splice elements such as BP, polypyrimidine tract and the 3' acceptor AG dinucleotides were deleted and replaced with random sequences (SEQ ID NO:85). This PTM still has 3' and 5' binding domains and the functional 5' splice site. PTM29 lacks the 2nd binding domain + 5' ss but still has the 3' binding domain 3' splice site, while PTM30 lacks the 1st binding domain + 3' splice site but has the functional 5' splice site and 2nd binding domain.

Paragraph 00190 beginning on page 74, line 21 and ending on page 75, line 4 has been **amended** as follows:

[00190] To examine the double-*trans*-splicing mediated restoration of β-gal function, 293T cells were either transfected with 2 Φg of target or PTM alone or co-transfected with 2 Φg of target + 1.5 Φg of PTM using Lipofectamine Plus reagent. 48 hrs. after transfection, total RNA was isolated and analyzed by RT-PCR using K1-1F and Lac-6R primers. These primers amplify both *cis*- and *trans*-spliced products in a single reaction which were identified based on the size. The *cis*-spliced product is 295 bp in size while the *trans*-spliced product is 230 bp in size. To confirm that *trans*-splicing between DSPTM7 and DSCFT1.6 pre-mRNA is precise, RT-PCR amplified products were excised, re-amplified using K1-2F and Lac-6R primers and sequenced directly using K1-2F or Lac-6R primers. As shown in Figure 23 *trans*-splicing occurred exactly at the predicted splice sites, confirming the precise internal exon substitution by two *trans*-splicing events (SEQ ID NO:86, 87).

Paragraph 00196 on page 78 has been **amended** as follows:

[00196] The repair model in Fig. 30 shows a portion of a target CFTR pre-mRNA consisting of exons 1-9, mini-intron 9, exon 10 containing the delta 508 mutation, mini-intron 10 and exons 11-24 (Fig. 30). The PTM shown in the figure consists of exon 10 coding sequences (containing codon 508) and two *trans*-splicing domains each with its own splicing elements (acceptor and donor sites, branchpoint and pyrimidine tract) and a binding domain complementary to intron 9 splice site, part of exon 10 (5' and 3' ends) and intron 10 5' splice site (SEQ ID NO:88) (Fig. 31 (DS-CF1)). Exon 10 of the PTM also has modified codon usage throughout to reduce antisense effects between exon 10 of the PTM and it's own binding domains and for PTMs that have binding domains which are complementary to exon sequences (Fig. 31). A double-*trans*-splicing event between the PTM and target should produce a repaired full-length mRNA.

Paragraph 00197 on page 78 has been **amended** as follows:

[00197] Fig. 32 shows the sequence of a single PCR product showing target exon 9 correctly spliced to PTM 20 exon 10 (with modified codons) (upper panel) (SEQ ID NO:89), codon 508 in exon 10 of the PTM (middle panel) (SEQ ID NO:90) and PTM exon 10 correctly spliced to target exon 11 (lower panel) (SEQ ID NO:91). The sequence of a repaired target was generated by RT-PCR followed by PCR.

Paragraph 00201 on page 80 has been **amended** as follows:

[00201] RT-PCR was performed using an EZ-RT-PCR kit (Perkin-Elmer, Foster, CA). Each reaction contained 0.03 to 1.0 Φg of total RNA and 80 ng of a 5' and 3' specific primer in a 40 Φl reaction volume. RT-PCR products were electrophoresed on 2% Seaken agarose gels. The PTM- and target-specific oligonucleotides used to generate *trans*-spliced products are 5'-CGCTGGAAAAACGAGCTTGTTG-3' (primer CF93) (SEQ ID NO:74) and 5'-ACTCAGTGTGATTCCACCTTCTC-3' (primer CF111) (SEQ ID NO:75) , respectively. The PTM- and target-specific oligonucleotides used to generate *cis*-spliced products were CF1 and CF93. The sequence of oligonucleotide CF1 is 5'-GACCTCTGCAGACTTCACTTCTAATGATGATTATGG-3' (SEQ ID NO:76).

Paragraph 00203 on page 81 has been **amended** as follows:

[00203] Figure 36 shows the sequence of *cis*- and *trans*-spliced products. The top panel of Fig. 36A shows target exon 10 with it's three missing nucleotides (CTT) (SEQ ID NO:93), whilst the lower panel shows exon 10 and 11 of the target correctly spliced together (SEQ ID NO:94). Figure 36B is a partial sequence of a single PCR product showing the modified codons in exon 10 of the PTM (upper panel) (SEQ ID NO:95), codon 508 in exon 10 of the PTM (middle panel) (SEQ ID NO:96), and PTM exon 10 correctly spliced to target exon 11 (lower panel) (SEQ ID NO:97), indicating that *trans*-splicing is accurate. The sequence of the repaired target was generated by RT-PCR followed by PCR.

Paragraph 00205 beginning on page 82, line 7 and ending on page 83, line 4 has been **amended** as follows:

[00205] Targets: pc3.1lacZCF9, pc3.1lacZCF9m, and pc3.1lacZHCG1m. pc3.1lacZCF9 encodes for a normal *lacZ* pre-mRNA was constructed using *lacZ* coding sequences nucleotides 1-1788 as 5' exon, CFTR mini-intron 9 followed by *lacZ* coding sequences nucleotides 1789-3174 as 3' exon. This is similar to pc3.1lacZ-T2 construct but without stop codons in the *lacZ* 3' exon and has CFTR mini-intron 9 instead of βHCG6 intron 1 (Fig. 37A). CFTR mini-intron 9 was PCR amplified using plasmid T5 as template and primers CFIN-9F (5'-CTAGGATCCCGTTCTTTTGTTCTTCACT ATTAA) (SEQ ID NO:77) and CFIN-9R (5'-CTAGGGTTACCGAAGTAAAACCATACTTATTAG, restriction sites underlined) (SEQ ID NO:78), digested with *BamH* I and *BstE* II and cloned in place of BHCG6 intron 1 of pc3.1lacZ-T2 plasmid. pc3.1lacZCF9m expresses a defective *lacZ* pre-mRNA and is identical to pc3.1lacZCF9 but contains two in-frame non-sense codons in the 3' exon (Fig. 37A). pc3.1lacZHCG1m is a chimeric target, which includes the *lacZ* 5' exon followed by intron 1 and exon 2 of βHCG6. This is similar to pc3.1lacZCF9m except that it contains exon 2 of βHCG6 in place of mutant *lacZ* 3' exon. βHCG6 exon 2 was PCR amplified using βHCG6 plasmid (accession # X00266) as template DNA and primers HCGEx-2F (5'-GCATGGTTACCCTGCAGGGGCTGCTGCTGTTGCTG) (SEQ ID NO:79) and HCGEx-2R (5'-CTGAAAGCTTGTTAACCAGCTCACCATGGTGGGGCAG, restriction sites underlined) (SEQ ID NO:80) digested with *BstE* II and *Hind* III and cloned in place of the *lacZ* 3' exon of pc3.1lacZCF9m. Plasmid pcDNA3.1/HisB/*lacZ* (Invitrogen, Carlsbad, CA) was used as DNA template to produce 5' and 3' *lacZ* exons. The *lacZ* 5' exon is 1788 bp long, has an ATG initiation codon, *lacZ* 3' exon (without stop codons) is 1385 bp long and has a transcription termination signal at the end of the 3' exon. CFTR mini-intron 9 and βHCG6 intron 1 are 548 bp

and 352 bp in size, respectively, and both have 5' and 3' splice signals. Exon 2 of βHCG6 is 162 bp long and has a transcription termination signal at the end of the exon.

Paragraph 00206 beginning on page 83, line 10 and ending on page 84, line 2 has been **amended** as follows:

[00206] Pre-trans-splicing Molecules (PTMs): PTM-CF14 is an identical version of pcPTM1 with minor modifications in the *trans*-splicing domain (Fig. 37B). PTM-CF14 is a linear version and contains a 23 bp antisense binding domain (BD) (5'-ACCCATCATTATTAGGTCATTAT) (SEQ ID NO:81) complementary to CFTR mini-intron 9, 18 bp spacer, a canonical branch point sequence (UACUAAC; BP) and an extended polypyrimidine tract (PPT) followed by normal *lacZ* 3' exon. PTM-CF22, PTM-CF24, PTM-CF26 and PTM-CF27 are identical to PTM-CF14 except they differ in length of the BD (Fig. 37B). sPTM-CF18 has a 32 bp BD, sPTM-CF22 and sPTM-CF24 contain the same BD as PTM-CF22 and PTM-CF24, respectively. In these PTMs, the binding domains were modified to create intra-molecular stem-loop structure ("safety") to mask the 3' splice-site of the PTM. Different binding domains were produced by PCR amplification using specific primers (with unique *Nhe* I and *Sac* II sites) and a plasmid containing CFTR mini-intron 9 as template. PCR products were digested with *Nhe* I and *Sac* II and cloned into a PTM plasmid consisting of spacer sequences, 3' splice elements (BP, PPT and acceptor AG dinucleotide) followed by a normal *lacZ* 3' exon.

Paragraph 00209 beginning on page 84, line 17 and ending on page 85, line 8 has been **amended** as follows:

[00209] RT-PCR was performed as suggested by manufacturer using an EZ *rTth* RNA PCR kit (Perkins-Elmer, Foster City, CA). A typical reaction (50 Φl) contained 25-500 ng of total RNA, 100 ng of 5' target specific primer (common to *cis*- and *trans*-spliced products) (Lac-9F, 5'-GATCAAATCTGTCGATCCTTCC) (SEQ ID NO:82) and 100 ng of 3' primer (Lac-3R, 5'-CTGATCCACCCAGTCCCATTA, target specific primer for *cis*-splicing (SEQ ID NO:83), and Lac-5R, 5'-GACTGATCCACCCAGTCCCAGA (SEQ ID NO:84), PTM specific primer for *trans*-splicing), 1X reverse transcription buffer (100 mM Tris-HCl, pH 8.3, 900 mM KCL with 1 mM $MnCl_2$), 200 ΦM dNTPs and 10 units of *rTth* DNA polymerase. RT reactions were performed at 60EC for 45 min. followed by 30 sec pre-heating at 94EC and 25-35 cycles of PCR amplification at 94EC for 18 sec, annealing and extension at 60EC for 1 min followed by a final extension at 70EC for 7 min. The reaction products were analyzed by agarose gel electrophoresis.

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Respectfully submitted,

Carmella L. Stephens

Rochelle K. Seide
PTO Registration No. 32,300

Carmella L. Stephens
PTO Registration No. 41,328
Attorney/Agent for Applicant

BAKER BOTTS, L.L.P.
30 Rockefeller Plaza
New York, NY 10112
(212) 408-2539

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New York, NY 10112
(212) 408-2539